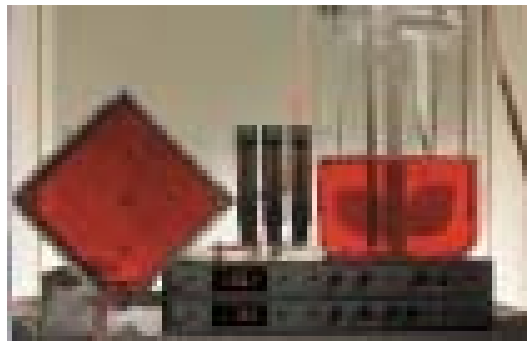
BioReliance®
invitrogen bioservices



Detection of Adventitious Viruses in Biologicals – A Rare Occurrence

Vaccine Cell Substrates 2004

June 30th, 2004

Raymond Nims

Rockville, Maryland

BioReliance®
invitrogen bioservices

Detection of Viruses in Biologicals Using Screening Assays - Conceptual Basis

Principle: Viruses will replicate in a suitable host cell, expressing their presence through cytopathic effects, and/or hemagglutination or hemadsorption of specific types of erythrocytes

BioReliance®
invitrogen bioservices

Indicator Cells Used in Adventitious Virus Testing

Indicator cell lines are selected on the basis of theoretical susceptibility to viruses of concern. In *in vitro* screening, a human diploid cell and a primate cell are used to detect viruses infectious for human cells, while a third indicator of a similar species as the cell substrate used in manufacture of the biological is used to detect viruses infectious for those cells.

In bovine and porcine virus testing, bovine and porcine indicator cells are employed.

BioReliance®
invitrogen bioservices

Regulatory Background for Raw Materials Testing

- In USA: 9 CFR 113.53
- In EU: ICH Viral Safety Documents Q5D (1993), Q5A(1997)

BioReliance
invitrogen bioservices

Regulatory Background for In Vitro Viral Screen

- In USA: Driven by the Points to Consider Documents (guidelines formulated by the FDA, not laws)
  - Characterization of Cell Lines Used to Produce Biologicals (1993)
  - Manufacture & Testing of Monoclonal Antibody Products (1997)
  - Guidance for Human Somatic Cell Therapy & Gene Therapy (1998)

- In EU: ICH Viral Safety Documents Q5D (1993), Q5A(1997), European Pharmacopoeia Commission 1999 Tests for Extraneous Agents in Viral Vaccines for Human Use.

BioReliance
invitrogen bioservices

Sampling Points For Adventitious Virus Testing

- Raw materials (serum, trypsin, other animal-derived materials) 9 CFR-compliant bovine and porcine virus screens
- Cell banks (WCB, MCB, EPC) In vitro virus screen, bovine or porcine virus screens
- Unprocessed bulk harvest (Lot Release) In vitro virus screen

BioReliance
invitrogen bioservices

Special Considerations for Vaccine Testing

- Cellular vaccine products may be evaluated in a manner similar to Cell Line Characterization
- Live viral vaccines (vaccinia, influenza, etc.) typically require neutralization with type-specific antisera prior to testing to avoid false positive effects in the screening assays.

BioReliance®
invitrogen bioservices

Sampling for Adventitious Virus Testing

Raw Materials:

- Serum (15% in medium)
- Trypsin (5 g, centrifuged, reconstituted in trypsin inhibitor
- Other animal-derived materials (3x nominal concentration)

Cell banks: 10^7 cells/ml in conditioned medium

Bulk harvests: sample size (3 ml per indicator cell line) has no relationship to total harvest volume.

BioReliance®
invitrogen bioservices

Methodology

1. Host cells are exposed to test agent in flasks or plates
2. Cells are incubated for 2-4 weeks and observed under inverted microscope
3. Test cultures are compared to negative and positive control cultures
4. At termination, additional endpoints may be performed (hemadsorption/hemagglutination of erythrocytes, Immunofluorescence)

BioReliance
invitrogen bioservices

Identification of a Contaminant

- Consideration of host range, cytopathic effect, hemadsorption/hemagglutination pattern
- Isolate remains infectious after passage to fresh cells?
- Conduct PCR testing for likely candidates
- Conduct IFA screening of infected cells
- Conduct Electron microscopy of infected cells

Detection of Viruses in Raw Materials

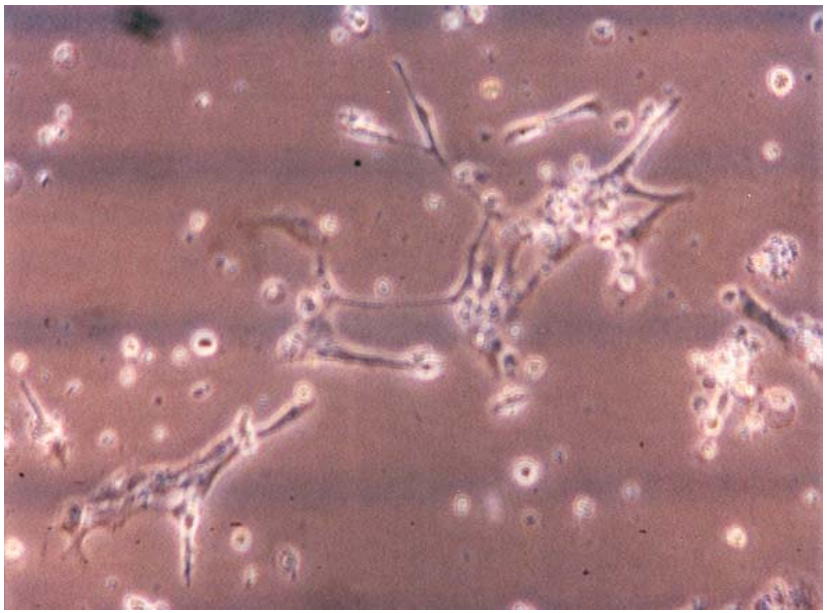
Raw Material	Viruses Detected
Bovine serum	BVDV bovine polyoma virus
Porcine trypsin	PPV

Detection of Viruses in Biologicals Using Screening Assays - A Very Rare Event

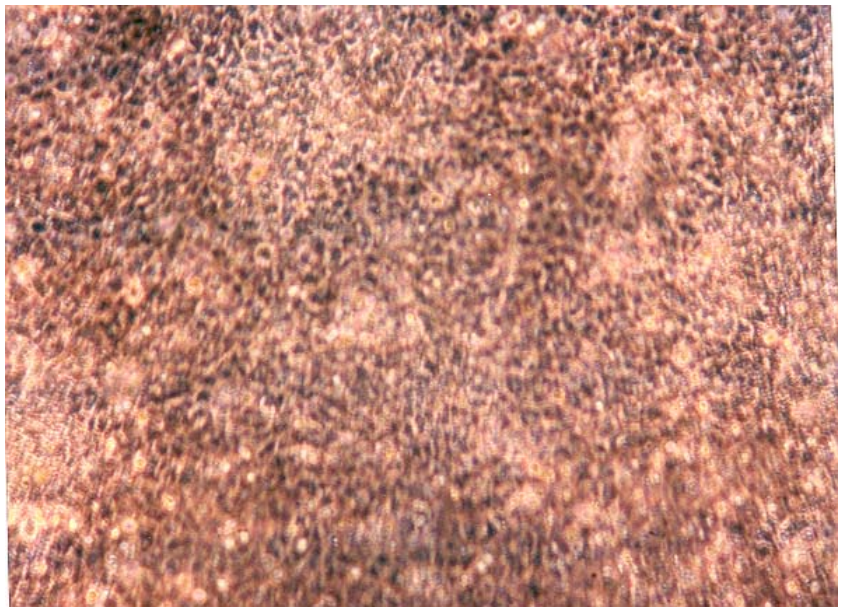
Biological Type	Viruses Detected
Cell Lines (MCB, WCB, EPC)	none
Gene Therapy Vectors	replication-competent adenovirus
Monoclonal Antibodies	none
Recombinant Proteins	
• non-CHO cell process	none
• CHO cell process	MMV, REO, Cache Valley Virus
Vaccines	none

BioReliance®
invitrogen bioservices

Morphological Changes in 324K Cells Infected with REO Virus

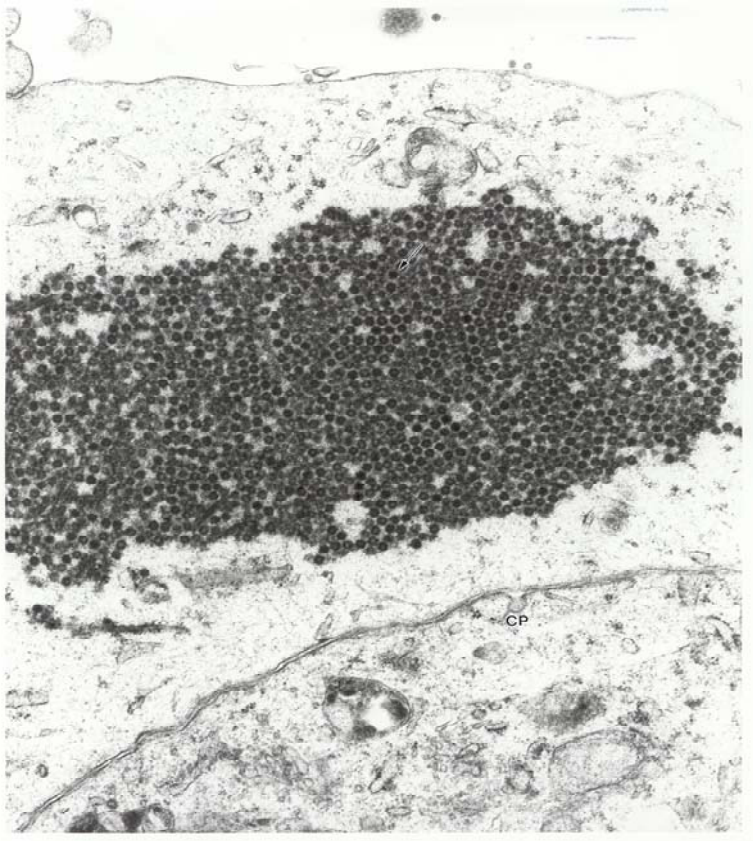


REO-infected 324K cells
Day 20 10x magnification



Control 324K cells
Day 20 10x magnification

Electron Micrograph of REO-Infected 324K Cells



- packing of viral particles in crystalline arrays near nucleus at late stage of infection
- 65-75 nm diameter spherical particles -with double concentric ring structure
- 37000x magnification

BioReliance®
invitrogen bioservices

Summary

- Raw materials testing can reduce potential for introduction of viruses in serum, trypsin, other animal-derived materials

- Adventitious virus screening is intended to detect gross contamination of raw materials, cell lines, and bulk harvests. Contaminants are rarely detected in cell substrates and bulk harvests. Information obtained from such occurrences is used to design cleaning and process (viral clearance) validation studies.